



SEQUENCE LISTING

<110> Kincaid, Randall L.
Angov, Evalina
Lyon, Jeffrey A.
Veritas, Inc.

<120> Improved Protein Expression by Codon Harmonization and
Translational Attenuation

<130> 016873-000400US

<140> US 10/677,641

<141> 2003-10-01

<150> US 60/369,741

<151> 2002-04-01

<150> US 60/379,688

<151> 2002-05-09

<150> US 60/425,719

<151> 2002-11-12

<150> WO PCT/US03/10384

<151> 2003-04-01

<160> 10

<170> PatentIn Ver. 2.1

<210> 1

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR
amplification primer FVO-PCR1

<400> 1

gggtcgggtac catggcagta actccttccg taattgat

38

<210> 2

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR
amplification primer FVO-PCR2

<400> 2

ggatcagatg cggccgctta actgcagaaa ataccatcga aaagtgga

48

<210> 3

<211> 23

<212> DNA

<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:PCR
 primer-directed mutagenesis overlapping
 oligonucleotide EA3

 <400> 3
 taaaaaatat ataaacgaca aac 23

 <210> 4
 <211> 23
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:PCR
 primer-directed mutagenesis overlapping
 oligonucleotide EA5

 <400> 4
 aaaaggaag atatttctca ttt 23

 <210> 5
 <211> 71
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:synthesized
 sense strand oligonucleotide EA485-CDFVO

 <400> 5
 cgcagttact ccattctgta ttgataatat tctttctaaa attgaaaacg aatatgaggt 60
 tttatatatta a 71

 <210> 6
 <211> 79
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:synthesized
 sense strand oligonucleotide EA493-CDFVO

 <400> 6
 gggttttaaat ataaaacctc atattcgttt tcaatttttag aaagaatatt atcaataaca 60
 gatggagtaa ctgcggtac 79

 <210> 7
 <211> 1065
 <212> DNA
 <213> Plasmodium falciparum

 <220>
 <223> native codon MSP1-42

<220>
 <221> CDS
 <222> (1)..(1065)
 <223> MSP1-42

<400> 7
 gca gta act cct tcc gta att gat aac ata ctt tct aaa att gaa aat 48
 Ala Val Thr Pro Ser Val Ile Asp Asn Ile Leu Ser Lys Ile Glu Asn
 1 5 10 15
 gaa tat gag gtt tta tat tta aaa cct tta gca ggt gtt tat aga agt 96
 Glu Tyr Glu Val Leu Tyr Leu Lys Pro Leu Ala Gly Val Tyr Arg Ser
 20 25 30
 tta aaa aaa caa tta gaa aat aac gtt atg aca ttt aat gtt aat gtt 144
 Leu Lys Lys Gln Leu Glu Asn Asn Val Met Thr Phe Asn Val Asn Val
 35 40 45
 aag gat att tta aat tca cga ttt aat aaa cgt gaa aat ttc aaa aat 192
 Lys Asp Ile Leu Asn Ser Arg Phe Asn Lys Arg Glu Asn Phe Lys Asn
 50 55 60
 gtt tta gaa tca gat tta att cca tat aaa gat tta aca tca agt aat 240
 Val Leu Glu Ser Asp Leu Ile Pro Tyr Lys Asp Leu Thr Ser Ser Asn
 65 70 75 80
 tat gtt gtc aaa gat cca tat aaa ttt ctt aat aaa gaa aaa aga gat 288
 Tyr Val Val Lys Asp Pro Tyr Lys Phe Leu Asn Lys Glu Lys Arg Asp
 85 90 95
 aaa ttc tta agc agt tat aat tat att aag gat tca ata gat acg gat 336
 Lys Phe Leu Ser Ser Tyr Asn Tyr Ile Lys Asp Ser Ile Asp Thr Asp
 100 105 110
 ata aat ttt gca aat gat gtt ctt gga tat tat aaa ata tta tcc gag 384
 Ile Asn Phe Ala Asn Asp Val Leu Gly Tyr Tyr Lys Ile Leu Ser Glu
 115 120 125
 aaa tat aaa tca gat tta gat tca att aaa aaa tat ata aac gac aaa 432
 Lys Tyr Lys Ser Asp Leu Asp Ser Ile Lys Lys Tyr Ile Asn Asp Lys
 130 135 140
 caa ggt gaa aat gag aaa tac ctt ccc ttt tta aac aat att gag acc 480
 Gln Gly Glu Asn Glu Lys Tyr Leu Pro Phe Leu Asn Asn Ile Glu Thr
 145 150 155 160
 tta tat aaa aca gtt aat gat aaa att gat tta ttt gta att cat tta 528
 Leu Tyr Lys Thr Val Asn Asp Lys Ile Asp Leu Phe Val Ile His Leu
 165 170 175
 gaa gca aaa gtt cta aat tat aca tat gag aaa tca aac gta gaa gtt 576
 Glu Ala Lys Val Leu Asn Tyr Thr Tyr Glu Lys Ser Asn Val Glu Val
 180 185 190
 aaa ata aaa gaa ctt aat tac tta aaa aca att caa gac aaa ttg gca 624
 Lys Ile Lys Glu Leu Asn Tyr Leu Lys Thr Ile Gln Asp Lys Leu Ala
 195 200 205
 gat ttt aaa aaa aat aac aat ttc gtt gga att gct gat tta tca aca 672
 Asp Phe Lys Lys Asn Asn Asn Phe Val Gly Ile Ala Asp Leu Ser Thr
 210 215 220

gat tat aac cat aat aac tta ttg aca aag ttc ctt agt aca ggt atg	720
Asp Tyr Asn His Asn Asn Leu Leu Thr Lys Phe Leu Ser Thr Gly Met	
225 230 235 240	
gtt ttt gaa aat ctc gct aaa acc gtt tta tct aat tta ctt gat gga	768
Val Phe Glu Asn Leu Ala Lys Thr Val Leu Ser Asn Leu Leu Asp Gly	
245 250 255	
aac ttg caa ggt atg tta aac att tca caa cac caa tgc gta aaa aaa	816
Asn Leu Gln Gly Met Leu Asn Ile Ser Gln His Gln Cys Val Lys Lys	
260 265 270	
caa tgt cca caa aat tct gga tgt ttc aga cat tta gat gaa aga gaa	864
Gln Cys Pro Gln Asn Ser Gly Cys Phe Arg His Leu Asp Glu Arg Glu	
275 280 285	
gaa tgt aaa tgt tta tta aat tac aaa caa gaa ggt gat aaa tgt gtt	912
Glu Cys Lys Cys Leu Leu Asn Tyr Lys Gln Glu Gly Asp Lys Cys Val	
290 295 300	
gaa aat cca aat cct act tgt aac gaa aat aat ggt gga tgt gat gca	960
Glu Asn Pro Asn Pro Thr Cys Asn Glu Asn Asn Gly Gly Cys Asp Ala	
305 310 315 320	
gat gcc aaa tgt acc gaa gaa gat tca ggt agc aac gga aag aaa atc	1008
Asp Ala Lys Cys Thr Glu Glu Asp Ser Gly Ser Asn Gly Lys Lys Ile	
325 330 335	
aca tgt gaa tgt act aaa cct gat tct tat cca ctt ttc gat ggt att	1056
Thr Cys Glu Cys Thr Lys Pro Asp Ser Tyr Pro Leu Phe Asp Gly Ile	
340 345 350	
ttc tgc agt	1065
Phe Cys Ser	
355	

<210> 8
 <211> 355
 <212> PRT
 <213> Plasmodium falciparum

<220>
 <223> native codon MSP1-42

<400> 8
 Ala Val Thr Pro Ser Val Ile Asp Asn Ile Leu Ser Lys Ile Glu Asn
 1 5 10 15
 Glu Tyr Glu Val Leu Tyr Leu Lys Pro Leu Ala Gly Val Tyr Arg Ser
 20 25 30
 Leu Lys Lys Gln Leu Glu Asn Asn Val Met Thr Phe Asn Val Asn Val
 35 40 45
 Lys Asp Ile Leu Asn Ser Arg Phe Asn Lys Arg Glu Asn Phe Lys Asn
 50 55 60
 Val Leu Glu Ser Asp Leu Ile Pro Tyr Lys Asp Leu Thr Ser Ser Asn
 65 70 75 80

Tyr	Val	Val	Lys	Asp	Pro	Tyr	Lys	Phe	Leu	Asn	Lys	Glu	Lys	Arg	Asp	
				85					90					95		
Lys	Phe	Leu	Ser	Ser	Tyr	Asn	Tyr	Ile	Lys	Asp	Ser	Ile	Asp	Thr	Asp	
			100					105					110			
Ile	Asn	Phe	Ala	Asn	Asp	Val	Leu	Gly	Tyr	Tyr	Lys	Ile	Leu	Ser	Glu	
		115					120					125				
Lys	Tyr	Lys	Ser	Asp	Leu	Asp	Ser	Ile	Lys	Lys	Tyr	Ile	Asn	Asp	Lys	
	130					135					140					
Gln	Gly	Glu	Asn	Glu	Lys	Tyr	Leu	Pro	Phe	Leu	Asn	Asn	Ile	Glu	Thr	
145					150					155					160	
Leu	Tyr	Lys	Thr	Val	Asn	Asp	Lys	Ile	Asp	Leu	Phe	Val	Ile	His	Leu	
				165					170					175		
Glu	Ala	Lys	Val	Leu	Asn	Tyr	Thr	Tyr	Glu	Lys	Ser	Asn	Val	Glu	Val	
			180					185					190			
Lys	Ile	Lys	Glu	Leu	Asn	Tyr	Leu	Lys	Thr	Ile	Gln	Asp	Lys	Leu	Ala	
	195						200					205				
Asp	Phe	Lys	Lys	Asn	Asn	Asn	Phe	Val	Gly	Ile	Ala	Asp	Leu	Ser	Thr	
	210					215					220					
Asp	Tyr	Asn	His	Asn	Asn	Leu	Leu	Thr	Lys	Phe	Leu	Ser	Thr	Gly	Met	
225					230					235					240	
Val	Phe	Glu	Asn	Leu	Ala	Lys	Thr	Val	Leu	Ser	Asn	Leu	Leu	Asp	Gly	
				245					250					255		
Asn	Leu	Gln	Gly	Met	Leu	Asn	Ile	Ser	Gln	His	Gln	Cys	Val	Lys	Lys	
			260					265					270			
Gln	Cys	Pro	Gln	Asn	Ser	Gly	Cys	Phe	Arg	His	Leu	Asp	Glu	Arg	Glu	
		275					280					285				
Glu	Cys	Lys	Cys	Leu	Leu	Asn	Tyr	Lys	Gln	Glu	Gly	Asp	Lys	Cys	Val	
	290					295					300					
Glu	Asn	Pro	Asn	Pro	Thr	Cys	Asn	Glu	Asn	Asn	Gly	Gly	Cys	Asp	Ala	
305					310					315					320	
Asp	Ala	Lys	Cys	Thr	Glu	Glu	Asp	Ser	Gly	Ser	Asn	Gly	Lys	Lys	Ile	
				325					330					335		
Thr	Cys	Glu	Cys	Thr	Lys	Pro	Asp	Ser	Tyr	Pro	Leu	Phe	Asp	Gly	Ile	
			340					345					350			
Phe	Cys	Ser														
		355														

<210> 9

<211> 1065

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Plasmodium
falciparum MSP1-42 with harmonized codons for
expression in E. coli cl. 2

<400> 9

```
gctgttacgc catcggttat tgacaatatc ctcagcaaaa ttgaaaacga atacgagggt 60
ctgtacctga aaccactggc tggcgtttac cgttctctga aaaaacagct ggaaaacaat 120
gttatgacct tcaacgttaa cgtaaaggac attctgaaca gccgcttcaa caaacgcgaa 180
aacttcaaaa acgttctgga aagcgacctg attccataca aagacctgac cagctctaac 240
tacgttgtca aagacccata caaattcctc aacaaagaaa aacgtgacaa attcctgtcg 300
tcttacaact acattaagga cagcatcgac acggacatca acttcgctaa cgacgttctc 360
ggctactaca aaatcctgtc ggagaaatag aaaagcgacc tggacagcat taaaaaatac 420
atcaatgata aacagggcga aaacgagaaa tatctccctt tcctgaataa cattgagacg 480
ctgtacaaaa ccgttaacga caaaattgac ctgttcgtta ttcacctgga agctaaagtt 540
ctcaactaca cctacgagaa aagcaatggt gaagttaaaa tcaaagaact caactatctg 600
aaaaccattc aggataaact cgctgacttc aaaaaaaaca ataacttcgt tggcattgct 660
gacctgagca ccgactacaa tcacaacaat ctgctcacca agttcctctc taccggcatg 720
gttttcgaaa acctcgctaa aacggttctg agcaacctgc tcgacggcaa tctccagggc 780
atgctgaata ttagccagca tcagtgtggt aaaaaacagt gccacagaa cagcggctgc 840
ttccgtcacc tggacgaacg tgaagaatgc aaatgcctgc tgaactataa acaggaaggc 900
gacaaatgcg ttgaaaaccc aaacccaacg tgcaatgaaa acaacggcgg ctgcgacgct 960
gacgccaat gcacggaaga agacagcggc tcgaatggca agaaaattac ctgcgaatgc 1020
acgaaaccag acagctaccc actcttcgac ggcattttct gtagt 1065
```

<210> 10

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:N-terminal
hexahistidine, His tag, affinity tag

<400> 10

His His His His His His

1

5